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"Nuts and Bolts" of Disease Tolerance

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Disease tolerance describes the ability of an infected host to limit disease severity without negatively impacting the causative pathogen. Bessede et al. (2014) show that the aryl hydrocarbon receptor is an essential component of disease tolerance during bacterial infection in mice.

The pathologic outcome of infection is revealed by the appearance of clinical symptoms, reflecting a more or less pronounced dysfunction of homeostasis in the infected host. Depending on the severity of disease, host reproductive capacity and survival—fitness—might eventually be compromised as well. It follows that host defense strategies against infection should share as a common endeavor the preservation of homeostasis and fitness. The prevailing strategy to achieve this goal is to eliminate the causative agent of disease, i.e., the pathogen, via immune-driven resistance mechanisms (Figure 1).

Host resistance mechanisms rely on the recognition of pathogens by germline-encoded pattern recognition receptors (PRR), activating the host innate immune system, which targets pathogens for destruction and/or expulsion (Figure 1). Activation of adaptive immunity provides a more specific, robust, and long-lasting protection mechanism against infection. Enhancing immune-driven resistance mechanisms, e.g., through vaccination, has proven to be an extremely efficient therapeutic strategy against infectious diseases, relieving mankind from the evolutionary constraints imposed by many pathogens. Presumably for this reason, we came to consider immune-driven resistance mechanisms as the only defense strategy that really matters when taking into consideration protection against infectious diseases. Reality, however, is probably more complex.

The study by Bessede et al. (2014) highlights the "relative cost" associated with immune-driven resistance mechanisms, as these become pathologic and contribute to disease severity, i.e., immunopathology (Figure 1). Bessede et al. (2014) show that this evolutionary trade-off is reduced via an immunoregulatory mechanism involving a stress-response pathway controlled by the aryl hydrocarbon receptor (AhR) and conferring disease tolerance to infection (Figure 1).

Disease tolerance is a concept that stems from observations made originally in the context of infection in plants and revealing that these can "tolerate" pathogens via a defense strategy that does not appear to reduce their pathogen load but instead limits the extent of tissue damage associated with infection (Schaefer, 1971). This defense strategy, coined as tolerance, remained in the literature for more than a century, as a specificity of host-pathogen interactions in plants (Schaefer, 1971). As it turns out, however, tolerance is an evolutionary conserved host defense strategy against infection that is shared by plants and animals, including insects, worms, mice, and most likely humans as well (Medzhitov et al., 2012). Disease tolerance is the term used to describe the same concept defined originally in the plant literature and referring to preservation of host fitness during infection, without concomitant reduction of pathogen load (Medzhitov et al., 2012). The mechanisms underlying disease tolerance in mammals remain poorly understood, being linked so far to stress-responsive pathways that limit the extent of tissue damage caused directly by pathogens or indirectly by host immune-mediated resistance mechanisms (Figure 1; Figueiredo et al., 2013; Jamieson et al., 2013; Larsen et al., 2010). Bessede et al. (2014) propose that the stress-response pathway regulated by AhR is critically involved in promoting disease tolerance to bacterial infections in mice.

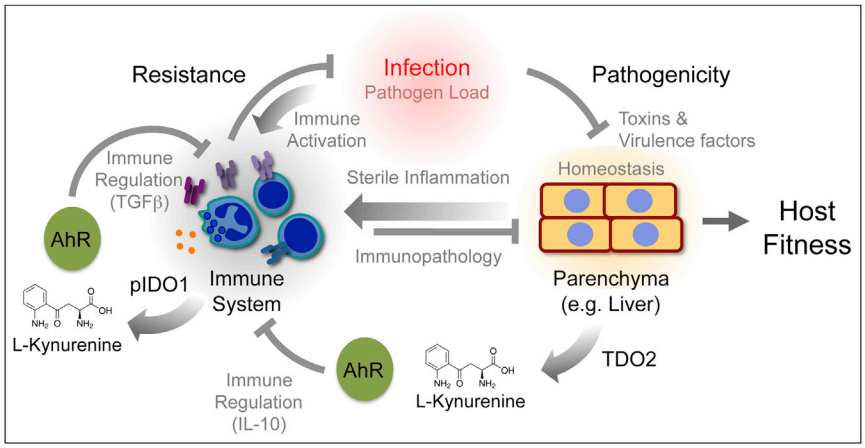

Figure 1. AhR and Disease Tolerance

Host soluble molecules and/or cells contributing to resistance mechanisms are labeled as the host immune system. Other host cells and/or soluble molecules, which do not exert a negative impact on pathogens, are labeled as the host parenchyma. Under homeostasis, fitness is governed to a large extent by parenchyma. During infection, however, toxins and other virulence factors expressed by pathogens can cause damage to the host parenchyma, driving homeostasis dysfunction and reducing host fitness. Although immune-driven resistance mechanisms confer protection against infection by reducing host pathogen load, they can also impose damage to the host parenchyma—immunopathology. This is exacerbated by desequestration of endogenous PRR agonists, associated with tissue damage, which promote sterile inflammation. Bessede et al. (2014) propose that L-kynurenine produced by TDO2 in the host parenchyma (e.g., liver) or by IDO1 within the immune system (e.g., macrophages and dendritic cells) is sensed by AhR, which regulates a stress-response exerting immunoregulatory effects that promote immune-driven resistance as well as disease tolerance. The immunoregulatory effect driven by TDO2 and AhR involves IL-10 while that driven by IDO1 and AhR involves TGF-β. Note that induction of AhR activity by L-kynurenine produced by TDO1 phosphorylates IDO1 (pIDO1) via the Scr kinase to produce more L-kynurenine and hence activate AhR further, forming a possible positive forward feedback loop.

AhR is an evolutionary conserved ligand-activated transcription factor, well known to toxicologists for its ability to sense xenobiotics—foreign molecules potentially toxic to the organism (Denison and Nagy, 2003). The dual action of AhR as a sensor and transcriptional regulator allows for a versatile stress response that provides cellular and systemic adaptation to xenobiotics (Hankinson, 1995). Over the years it has become apparent that AhR also senses endogenous ligands but the identification and physiologic relevance of these molecules has remained elusive. Moreover, several studies have highlighted that AhR exerts immunoregulatory effects that restrain the pathogenesis of immune-mediated inflammatory conditions, including infectious diseases (Stockinger et al., 2014). The findings now reported by Bessede et al. (2014) are in keeping with previous studies, revealing that L-kynurenine is a physiologic AhR agonist, required to support the protective effect of AhR against infectious diseases.

L-kynurenine is an endogenous product of tryptophan catabolism, generated physiologically in mammals by the tryptophan 2,3-dioxygenase (TDO or TDO2) as well as by indoleamine 2,3-dioxygenases (IDO) 1 and 2, the latter being well known for their immunoregulatory effects (Mellor and Munn, 2004). Using a genetic loss-of-function approach in mice, Bessede et al. (2014) demonstrate that when expressed under physiologic conditions, AhR and TDO2 are both required to confer host protection against endotoxic shock. This salutary effect is dependent on L-kynurenine, generated via TDO2 activity, with exogenous L-kynurenine administration bypassing the requirement for TDO2 but not for AhR expression and/or activity. The notion that tryptophan catabolism supports functionally the protective effect of AhR is further strengthened by the demonstration that L-kynurenine is indeed an AhR ligand. Considering that disruption of homeostasis associated with endotoxic shock is driven by the engagement of the PRR toll-like receptor 4 (TLR4) by the bacterial ligand lypopolysaccharide (LPS), in the absence of living bacteria, the protective effect of AhR and TDO2 is likely to participate in a disease tolerance pathway, as claimed.

Bessede et al. (2014) assessed whether this disease tolerance pathway is involved in LPS (endotoxin) tolerance, a phenomenon familiar to immunologists, in which a low level of TLR4 engagement confers protection against subsequent exposure to a lethal dose of LPS causing endotoxic shock. They found that AhR and IDO1 are both required for LPS tolerance in mice. Exogenous L-kynurenine administration bypasses the need for IDO1 but not for AhR expression and/or activity, suggesting that in this context AhR senses L-kynurenine produced mainly by IDO1. Presumably during the course of an infection, AhR senses L-kynurenine produced initially by TDO2, which activates AhR. This promotes the phosphorylation of IDO1, through a mechanism involving the Src kinase, and leading to the production of L-kynurenine, sustaining AhR activation. This argues for the establishment of a positive feed forward loop in which AhR activation induces the generation of L-kynurenine, via IDO1 activity, engaging further AhR to promote LPS tolerance.

Bessede et al. (2014) used a similar experimental approach to establish that LPS tolerance confers protection against bacterial infections, possibly via the AhR and L-kynurenine pathway. This is shown to be the case for *Salmonella* Typhimurium and *Streptococcus* infection in mice, inferring that LPS tolerance confers disease tolerance to bacterial infections. However, this protective effect is associated with a significant decrease in host pathogen load in both experimental models of infection tested. This argues that although critical to preserve host homeostasis and fitness during bacterial infection, LPS tolerance enhances not only disease tolerance but also resistance to infection, presumably acting via AhR and L-kynurenine. The latter was not demonstrated unequivocally for live bacteria.

This study by Bessede et al. (2014) also raises several questions possibly leading to future lines of research. AhR-deficient mice appear to be far more susceptible to endotoxic shock, as compared to TDO2-, IDO1-, or IDO2-deficient mice. This would argue that the protective effect

exerted by AhR is somehow more prevalent than the one exerted by enzymes generating its physiologic ligands, e.g., L-kynurenine. There are at least two possible and non-mutually exclusive explanations for this. The first is that the enzymes involved in tryptophan catabolism are to some extent redundant in their ability to produce the AhR ligand L-kynurenine. Alternatively, it is possible that additional AhR ligand(s) are produced physiologically via other host catabolic pathways that support the protective effects of AhR. Candidate AhR ligands include those produced by heme oxygenases (HOs), a stress-responsive enzyme that confers disease tolerance to polymicrobial infection (Larsen et al., 2010). Heme catabolism by HOs produces several putative AhR ligands including biliverdin (a direct end-product of HO activity) and bilirubin (a potent antioxidant generated from biliverdin catabolism by biliverdin reductase that activates AhR) (Denison and Nagy, 2003). Moreover, heme catabolism by HOs also generates carbon monoxide, a gasotransmitter that can bind ferrous (Fe^{2+}) iron contained in the heme group of AhR and modulate its activity. Whether regulation of disease tolerance by AhR acts via a mechanism involving the putative action of different end products of heme catabolism by HOs has not been established. If proven correct, this would argue for the integration of the AhR signal transduction pathways in a wider network of stress-responsive signaling pathways regulating disease tolerance to infection.

Although simple in its essence, the concept of disease tolerance should have major implications to our current understanding of the pathogenesis of infectious diseases. The study by Bessede et al. (2014) and future studies should provide the mechanistic insight, i.e., "nuts and bolts," allowing for targeting this defense strategy therapeutically toward a much-needed supplement to the current clinical approaches available in the treatment of infectious diseases.

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The Primordial Thymus: Everything You Need Under One Roof

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Lymphocytes normally develop within anatomically distinct tissues. In *Cell Reports*, Swann et al. (2014) reconstruct the primordial thymus and suggest that it was a site of combined T and B lymphopoiesis before evolving into an organ specialized for T cell production.

Through random recombination of gene segments encoding antigen receptors, lymphocytes recognize a wide range of pathogens and represent key players in adaptive immunity. They are also heterogeneous: B cells produce antibodies recognizing antigen in its native form, while αβ T cells recognize antigenic peptides via major histocompatibility complexes (MHC). In vertebrates, this lymphocyte heterogeneity is mirrored in the tissues that support their generation. Thus, bursectomy and thymectomy experiments in birds showed antibody-producing cells, and cytotoxic lymphocytes arose in anatomically distinct sites (Cooper et al., 1966). Significantly, studies on jawless vertebrates show that the specialized T lymphopoietic role of the thymus is ancient. For example, epithelial regions of developing lamprey gill structures express *Foxn1* (Bajoghli et al., 2011) encoding a transcription factor essential for thymic epithelial cell (TEC) development. Moreover, these tissues contained lymphocytes with features of T cells (e.g., expression of variable